

SOIL FUNGI IN MATURE AND CLEAR-CUT
CONIFEROUS FORESTS

ERLAND BÅÅTH



LUND 1980



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av

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AKADEMISK AVHANDLING

som för avläggande av filosofie doktorsexamen
vid matematisk-naturvetenskapliga fakulteten
vid universitetet i Lund kommer att offentligen
försvaras vid Mikrobiologiska institutionen,
hörsalen, Sölvegatan 21, torsdagen den 20 november,
kl. 10.00.



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This dissertation is a summary of the following communications:

- I. Bååth, E. and Söderström, B. 1979. Fungal biomass and fungal immobilization of plant nutrients in Swedish coniferous forest soils. *Revue d'Écologie et de Biologie du Sol* 16: 477-489.
- II. Söderström, B.E. and Bååth, E. 1978. Soil microfungi in three Swedish coniferous forests. *Holarctic Ecology* 1:62-72.
- III. Bååth, E. 1980. Soil fungal biomass after clear-cutting of a pine forest in Central Sweden. *Soil Biology and Biochemistry* (In press).
- IV. Bååth, E. Microfungi in a clear-cut pine forest soil in Central Sweden. Submitted to *Canadian Journal of Botany*.
- V. Bååth, E. and Söderström, B. 1979. The significance of hyphal diameter in calculation of fungal biovolume. *Oikos* 33:11-14.
- VI. Bååth, E. and Söderström, B. 1980. Comparisons of the agar-film and membrane-filter methods for the estimation of hyphal lengths in soil, with particular reference to the effect of magnification. *Soil Biology and Biochemistry* 12:385-387.

These papers will be referred to by Roman numerals I-VI in the text.

1. INTRODUCTION

Clear-cutting is the most common cutting method in Sweden nowadays and between 5 and 10 % of the land normally covered with forests is at present clear-cut area (Nilsson 1974). The biological consequences of such an operation are, however, poorly understood. This includes the effect on soil fungi.

This thesis deals with the fungal community in coniferous forest soil, with emphasis on fungal biomass and microfungial species composition. The main purpose was to compare the situation before and after a clear-cutting, but fungi were also studied in different forests. Papers I and II describe the situation in mature forests, while the effects of clear-cutting are mainly dealt with in papers III and IV. During the course of these investigations several methodological problems were encountered, some of which are dealt with in papers V and VI.

2. THE SITES

Seven sites in four different research areas have been studied. Four of the sites were situated at Ivantjärnsheden (Jädraås, Gästrikland, Central Sweden). This is the main research area of the Swedish Coniferous Forest Project. It is a pure Scots pine (Pinus silvestris L.) forest, with an undervegetation dominated by heather (Calluna vulgaris (L.) Hull), cowberry (Vaccinium vitis-idaea L.), mosses (mainly feather mosses, Hylocomium splendens (Hedw.) B.S.G.) and lichens (Cladonia spp.). The three other sites studied were a Norway spruce (Picea abies (L.) Karst) forest planted on a former beech

forest soil in Skåne, South Sweden, a mixed coniferous forest in Dalarna, Central Sweden, and a Scots pine forest in Västerbotten, North Sweden.

At Ivantjärnsheden the work was concentrated on two of the sites, designated Ih 0 and Ih VA. Both were about 120-yr old forests at the beginning of the investigation and were situated only 500 m apart. Ih VA was used as a reference stand. Ih 0 was clear-cut in late winter 1976. The site was divided into two treatments. In one the felling residues were removed, while in the other twice the normal amounts of slash were left on the ground. The effect of clear-cutting was also studied in an adjacent site (Ih I), clear-cut appr. 10 years earlier.

The clear-cutting of Ih 0 dramatically changed the vegetation. Apart from the removal of the trees, the field layer was also affected. Almost no regrowth of pines, grass, herbs or bushes was found at Ih 0 during the study. Chemical and physical soil variables were also changed, e.g. temperature and soil moisture varied to a greater extent, especially in the treatment where the slash had been removed.

The typical soil in Swedish coniferous forests is podzolic. The upper part of this type of soil consists mainly of material of organic origin, while the lower part mainly consists of mineral particles. These two parts of the soil, the organic and the mineral, are further differentiated into different soil horizons (see I and III for a description of these in more detail).

3. METHODS

Total fungal length was determined with the agar-film method (Jones & Mollison 1948). The original method was slightly modified (I). This method does not distinguish between active and inactive fungal hyphae, but has been considered to be the best and most accurate technique for total fungal length measurements (Nicholas & Parkinson 1967).

The FDA-active fungal length was determined with the fluorescein diacetate (FDA) method (Söderström 1977). This method was considered to give a better estimation of the active part of the soil hyphae and thus more sensitive to changes in fungal growth (Söderström 1979a). The method underestimates the active fungal length, however, as shown by Söderström (1979b).

For the conversion of fungal biovolume to fungal biomass and to fungal content of different nutrients, conversion factors for a number of hyphal properties like density and percentage dry weight of wet weight must be used. Radically different values for these factors are to be found in the literature. Furthermore, most of these values are derived from pure culture studies on laboratory media and are therefore difficult to relate to the natural situation. The biomass figures, as well as those for fungal immobilization of different plant nutrients given in papers I and III therefore only indicate the order of magnitude and are not to be considered as absolute values. For a comparative purpose between different horizons or between different sites, the values ought, however, to be more reliable.

Microfungi were isolated with a soil washing technique (Parkinson & Williams 1961). In total about 8000 isolates, assigned to about 145 taxa, were found (II,IV). The technique used is considered to favour the isolation of fungi from hyphal fragments at the expense of spores and conidia, as these to a large extent are removed by the washing procedure. After washing, soil particles were plated onto carboxymethyl cellulose agar (Gams 1960). This substrate allows a wide variety of microfungi to grow with a rather thin growth. This facilitates the isolation of slow growing, late sporulating fungi. Details on the isolation procedure are given in paper II.

4. FUNGI IN MATURE FORESTS (I,II,III,IV)

The amount of total fungal hyphae in Swedish coniferous forest soils was large compared with other investigations in similar forests (see I for references). The greatest amounts of hyphae per g dry soil were always found in the organic horizons, and considerably less was found in the mineral horizons. However, calculated on a square meter basis, the amount of fungi in the mineral soil equaled that in the organic horizons.

There was a great variation between the different sites studied. The total hyphal length ranged between 2 900 and 66 900 m·g dw^{-1} in the organic soil layer and between 140 and 2 100 m·g dw^{-1} in the mineral soil.

Qualitative differences between the different soil layers were detected. The proportion of hyphae with clamp connections (basidiomycete hyphae) differed for example between the different horizons in one

of the sites. The mean hyphal diameter also varied usually being greater in the organic soil as compared to the deeper soil layers.

When the total fungal biomass was expressed per square meter, 73 and 102 g dw·m⁻² respectively were found at two of the sites studied. Using these biomass values, the fungal immobilization of some plant nutrients was estimated. The greatest immobilization for nitrogen and phosphorus was found in the organic soil horizon in one site, while no differences between the horizons were found in the other.

The microfungal species composition in the three forest soils studied was in accordance with other investigations from temperate forest soils (Lucarotti et al. 1978, Widden 1979, see II and Söderström 1975 for further references). The most frequently isolated genera were Mortierella and Penicillium, which together accounted for between 43.5 and 60.1 % of the total number of isolates at any site studied. Mortierella was found throughout the soil profile, while Penicillium was almost totally restricted to the organic soil layers. Other genera with high frequency of isolation are listed in Tab.4, paper II. A complete list of isolated species is given in the appendix of paper II and in paper IV.

The species composition differed between the different soil layers. The difference was most pronounced between the organic and the mineral soil, while the microfungal community was more similar, when the soil layers within these two parts of the soil were compared. The colonization index (i.e. the number of isolates per plated particle) also changed with depth of the soil, normally being lower in the deeper soil horizons. The species diversity did not differ

to any great extent between the different soil horizons.

The fungal flora of the different sites was similar, especially if one and the same horizon was compared. Differences existed, however, and these were most pronounced between the planted spruce forest in the south of Sweden and the two sites in Central Sweden.

5. FUNGI AFTER CLEAR-CUTTING (III,IV)

Both the FDA-active and the total fungal biomass decreased after clear-cutting. This was evident in both treatments (with and without slash), as well as in all soil horizons. The FDA-active fungal content of the mineral soil appeared to decrease more than that of the organic soil. Calculated on a square meter basis (i.e. all soil layers were taken into account) the total biomass decreased from 80 to about $50 \text{ g dw} \cdot \text{m}^{-2}$ after three years. The FDA-active fungal biomass decreased from about 2 to about $1.5 \text{ g dw} \cdot \text{m}^{-2}$ during the same time. The fungal content of the older clear-cut area was still lower, indicating that the decrease in fungal biomass due to clear-cutting will continue for several years more.

Areas with slash generally had higher amounts of FDA-active fungi compared to areas without slash. In the organic horizon this difference was most pronounced immediately after the clear-cutting. Later the difference became less pronounced.

The micro-fungal species composition appeared only slightly affected after the clear-cutting. Principal component analysis (PCA)

indicated, however, that the fungal flora of the organic soil layers of the treatment without slash was changing, becoming more like that in the old clear-cut area. In the mineral soil and in the organic soil layers of the treatment with slash, PCA did not reveal any effect of clear-cutting.

As far as different species were concerned, the most noteworthy finding was Penicillium brevi-compactum. This species was seldom isolated in the mature forest. After the clear-cutting it was found more often and in the old clear-cut area 18 % of all isolates in the A₀₁ horizon was P. brevi-compactum.

The species diversity appeared to decrease in the organic soil horizons after clear-cutting, while no significant effect was found in the mineral soil. The number of isolates per plated particle was not affected by the silvicultural operation in any of the horizons studied.

6. METHODOLOGICAL PROBLEMS (V,VI)

The agar-film method has been commonly used for a long time in fungal biomass studies. Hansen et al. (1974) described a membrane filter method which was further modified by Sundman & Sivelä (1978). This method has the advantage that the preparation procedure is less time consuming than that of the agar-film technique. However, when the two methods were compared, the latter method consistently gave higher counts. The two methods were well correlated.

The optical system is very crucial when fungal length measurements are to be made microscopically. Frankland (1974) pointed out

the importance of using phase contrast optics. The magnification used is also of utmost importance. The use of 1250x total magnification gave values nearly twice as high compared to those obtained using 500x and 800x magnification. The measured mean hyphal diameter was also affected.

The calculation of the mean hyphal cross section can be done in different ways with the mode of calculation influencing the determined biovolume. The use of mean hyphal diameter underestimates the biovolume. The most accurate method takes into account the fact that hyphae with very different diameters dwell in the soil and that different hyphae fragment to a different degree during the preparation procedure. However, this mode of calculation is very time consuming. A more simple method, that gave somewhat lower results, was therefore used when the biovolume or the biomass of the different sites was compared (I,III). Depending on the mode of calculation the biovolume at one site was estimated to be 370, 440 or $520 \text{ cm}^3 \cdot \text{m}^{-2}$.

7. GENERAL COMMENTS

Clear-cutting is a very dramatic operation, which affects the environment considerably. A number of environmental factors are changed, which in turn influence the soil fungi. Changes in the root dynamics are suggested to be an important factor determining the registered decrease in fungal biomass after clear-cutting. A reduced root production and root exudation affect the saprophytic fungal flora and the mycorrhizal fungi lose their nutritional base when the trees are cut. The nutrient input to the soil from root litter equals the nutrient input from above ground litter in this type of conifer

ferous forest (Staaf & Berg 1977), a fact that stresses the importance of roots in this system.

The nutrient factor is obviously a very important factor in determining the amount of fungal hyphae in the soil. This factor could also explain the vertical distribution. The low fungal biomass in the mineral soil is probably directly related to the low organic matter content there.

With regard to the species composition the situation is less clear. One reason for this is our poor knowledge of the ecophysiology of many soil microfungi. Most such information is furthermore inferred from pure culture studies. This is somewhat hazardous, since pure culture studies are difficult to relate to the natural situation. Another reason is that as yet many soil microfungi have not been described or are impossible to assign to a specific species with any certainty. This includes atypical forms of common soil fungi as well as isolates, which remain sterile in pure culture and thus cannot be identified.

The diversity of the microfungal community in a podzol is low compared with for example that in an agricultural soil. This can be demonstrated if the results of paper IV and a similar investigation in an arable soil (Gams & Domsch 1967) are compared. Depending on the horizon studied between 40 and 55 different species were found in the first 500 isolates from the podzolic soil (IV, Fig.3). Gams & Domsch in their study found almost twice as many species, about 90 (calculated from their Fig.4).

A number of methods are available for the study of soil micro-organisms. These include different kinds of microscopical counting methods, like the ones used in this investigation, physiological methods (CO_2 , ATP etc.) and enzyme activity measurements. Comparison of different methods have shown that they often give very different results (VI, Paul & Johnson 1977, Domsch et al. 1979, Spiers & McGill 1979). At present no single method can be judged superior and as that always giving the most reliable result. The purpose of the investigation, the type of material studied and the laboratory facilities therefore must be taken into account when method(s) for a study are chosen.

The influence of man-made manipulations of the environment on soil fungi has in the present investigation been exemplified with clear-cutting. Changes in fungal biomass or species structure have been found in many other cases, e.g. after strip-mining (Lawrey 1977), soil burning (Widden & Parkinson 1975), artificial acid rain (Bååth et al. 1979), forest fertilization (E.Bååth, B.Lundgren and B.Söderström, unpublished) and heavy metal pollution (Jordan & Lechevallier 1975, A.Nordgren, unpublished). Soil fungi are an important area of study not only with regard to their importance in the decomposition processes in the soil, but also in that their study can contribute to the understanding and exposure of the impact of man on different ecosystems.

8. ACKNOWLEDGEMENTS

I am much indebted to Professor Börje Norén, Uppsala, who initiated my work and supported me in every way. Dr. Bengt Söderström, Lund, has during all this work given me inspiration and encouragement and Mr. Björn Lundgren, Lund, has been of much help during field work and discussions. I also wish to thank Dr. Walter Gams, Baarn, Holland, Dr. Birgit Hertz and Professor Claes Weibull, Lund. The skillful technical assistance of Mrs. Gunilla Wernerheim, Mrs. Ann-Margret Sonnerfelt and Mr. Leif Rilfors is greatly appreciated.

I furthermore wish to express my gratitude to all colleagues and friends at the Department of Microbial Ecology and the Department of Microbiology, Lund, as well as to those who worked within the Swedish Coniferous Forest Project, Uppsala.

Most of this work was done within the Swedish Coniferous Forest Project supported by the Swedish Natural Science Research Council and the Swedish Environmental Protection Board.

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